can also be used on the biopsy tissue to detect viral infection.

Although the histologic patterns have been described individually, rejection, ischemia, and viral infections can occur simultaneously, with each contributing to a varying degree to the extent of liver injury.

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The Molecular Pathology of Prion Diseases

NEW PATHOGENETIC MECHANISMS are being discovered for the group of central nervous system (CNS) diseases that includes Creutzfeldt-Jakob disease, the Gerstmann-Straussler syndrome, and kuru in humans and scrapie in animals. These disorders have been grouped together because they have similar clinical, genetic, and histologic features—spongiform degeneration of neurons, astrocytic gliosis, and amyloid plaque formation—and because each is infectious. Originally it was postulated that they were caused by an atypical virus because of the small size of the infectious particle predicted from experimental studies and the absence of histopathologic features characteristic of viral infections. That possibility, however, is now considered unlikely because of studies at the University of California at San Francisco showing that highly purified preparations of the scrapie agent consist almost entirely of a single protein and no detectable nucleic acid. For this reason, the smallest infectious particle was termed a "prion" and the protein associated with infectivity was termed the "prion protein."

Although the structure of the prion and the precise role of the prion protein in infectivity are not known, it has been learned that the prion protein is an abnormal derivative of a normally expressed CNS nerve cell sialoglycolipoprotein. The normal isoform is synthesized continuously in hamster nerve cells, is highly susceptible to proteinase K digestion, and turns over rapidly. In contrast, the abnormal isoform resists proteinase K digestion, turns over slowly, and accumulates in the brain during scrapie. Moreover, the abnormal isoform of the prion protein accumulates precisely in those regions of the gray matter where spongiform degeneration of nerve cell processes and reactive astrocytic gliosis are greatest. It also polymerizes into the filaments that form the amyloid plaques characteristic of these diseases.

Evidence indicates that a human isoform of the scrapie prion protein causes Creutzfeldt-Jakob disease, the Gerstmann-Straussler syndrome, and kuru. Abnormal proteinase-resistant forms of the prion protein, which react specifically with anti-hamster prion protein antibodies, have been found in the brains of patients with Creutzfeldt-Jakob disease but not in normal brains or those of patients with Alzheimer's disease. The amyloid plaques in the brains of patients with Creutzfeldt-Jakob disease and those with the Gerstmann-Straussler syndrome react specifically with anti-hamster scrapie prion protein antibodies. Also, human chromosomes contain a single-copy prion protein gene that has been localized to chromosome 20. In addition, the amino acid sequence of the human prion protein is 90% homologous to the hamster and mouse prion protein. For these reasons, scrapie, Creutzfeldt-Jakob disease, the Gerstmann-Straussler syndrome, and kuru have been grouped under the term "prion disease."

The central pathogenic event in prion diseases appears to be the abnormal metabolism and accumulation of a normally expressed nerve cell protein. The unique feature of prion diseases is that the abnormal prion protein appears to participate in transmitting the disease. The prion hypothesis is consistent with the failure to find a virus and with the absence of an inflammatory or immune response. It is genetically consistent because although most cases of Creutzfeldt-Jakob disease are sporadic, 10% are dominantly inherited, and virtually all cases of the Gerstmann-Straussler syndrome are dominantly inherited. Furthermore, the scrapie incubation time gene is genetically linked to the prion protein gene in mice. Therefore, except for infectivity, most of the features of these diseases are difficult to reconcile with the viral hypothesis.

Prion research is now focused on determining the chemical differences between the normal and abnormal isoforms of the prion protein; whether the abnormal prion protein, either alone or with a cofactor, is the transmissible agent; and the molecular mechanisms by which prions induce disease.

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Polymerase Chain Reaction—A Novel Method for Analyzing Specific DNA Sequences

THE ANALYSIS of DNA is becoming increasingly important in the study and diagnosis of hereditary, neoplastic, and infectious diseases. Often, however, the heterogeneity or small quantity of the sample available can limit the usefulness of conventional techniques. The polymerase chain reaction is a new enzymatic method for selectively replicating a specific nucleic acid sequence up to several hundred nucleotides in length within a complex mixture to facilitate its analysis.

To use the polymerase chain reaction procedure, the DNA sequences flanking the region of interest must be known. Two short oligonucleotide primers complementary to each of the two flanking regions but on opposite DNA strands are synthesized. These primers are added in vast molar excess to the sample DNA, which is then denatured and allowed to anneal to the primers. In the presence of deoxyribonucleoside triphosphates, a thermostable form of bacterial DNA polymerase uses each oligonucleotide as a primer to synthesize a copy of the adjacent DNA strand; each newly synthesized strand then provides a new template for synthesis from the opposite primer. By repeated cycles of denaturation, annealing, and synthesis, the region between the two primers is amplified exponentially. A 220,000-fold amplification of the desired region can be achieved after 20 such cycles; the amplified sequence can then be analyzed by a variety of techniques. Automated instrumentation capable of pro-

cessing many specimens for the polymerase chain reaction has recently become available.

The speed and sensitivity of the reaction procedure offer advantages for both prenatal and microbiologic diagnosis. The method has been applied to the prenatal diagnosis of sickle cell anemia and will find wider application as the genetic defects underlying other familial disorders are identified. It is also capable of detecting minute quantities of viral DNA in clinical specimens even before seroconversion occurs. The polymerase chain reaction has already proved useful in research laboratories for detecting mutations of cellular proto-oncogenes that are thought to play a role in human carcinogenesis; it may soon be used clinically to characterize these mutations in individual patients.

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Fine-Needle Aspiration Biopsy in the Diagnosis of Lymphadenopathy in Persons at Risk for AIDS

LYMPHADENOPATHY is a common finding in the acquired immunodeficiency syndrome (AIDS) and in the AIDS-related complex (ARC). Although this lymphadenopathy usually has a restricted differential diagnosis, it can be difficult to establish the precise cause of the nodal enlargement by history, physical examination, radiographic studies, and laboratory tests.

At the San Francisco General Hospital and Medical Center, we have found fine-needle aspiration (FNA) biopsy to be an accurate, well-tolerated, cost-effective, and useful method to initially evaluate lymphadenopathy in patients with AIDS or ARC. We have done more than 120 FNA biopsies of lymph nodes in such patients.

In our experience, about half the lymph node biopsy specimens in such patients show lymphoid hyperplasia. The other half reveal non-Hodgkin's lymphoma, mycobacterial infection, Kaposi's sarcoma, Hodgkin's disease, and various metastatic tumors. The smears showing lymphoid hyperplasia are characterized by a pleomorphic population of lymphocytes, histiocytes, polymorphonuclear leukocytes, plasma cells, and other lymphoid elements. The smears showing non-Hodgkin's lymphoma are characterized by a monomorphic population of abnormal lymphoid cells; we have further classified these cases as diffuse large cell, large cell immunoblastic, and small noncleaved lymphomas. The smears of patients with mycobacterial infections have consisted of histiocytes with thousands of intracytoplasmic organisms. The smears showing Kaposi's sarcoma have clusters of bland spindle cells not associated with inflammatory elements.

Falsely abnormal results ("false-positives") of FNA biopsies of lymph nodes in this group of patients did not occur in our series, but falsely normal ("false-negatives") results can occur. Possible reasons for false-negative results include sampling errors in lymph nodes with focal disease, taking a biopsy of a benign lymph node in a patient with abnormal nodes elsewhere, and error in microscopic interpretation. Because false-negative FNA biopsies can occur, it is imperative that clinicians using this test realize that a benign result does not entirely rule out involvement of the lymph node by a malignant or infectious process.

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DNA Fingerprinting—Applications for Resolving Medical, Legal, and Criminal Issues

THERE IS NOW a genetic test to determine individual identity. This DNA test or "DNA fingerprinting" holds the same standard of certainty as a set of fingerprints. The test exploits the occurrence of tandem-repetitive regions of DNA—minisatellites—which are scattered throughout the human genome. The minisatellites are highly polymorphic or hypervariable, resulting from unequal exchanges that vary the number of short repeat units in a minisatellite. Researchers at Leicester University, England, isolated three human minisatellite DNA fragments by molecular cloning, each containing tandem repeats of closely related variants of a short consensus sequence. Using these cloned DNA fragments as probes to detect the homologous sequences in the restriction endonuclease-digested human DNA, they discovered DNA banding patterns ("fingerprints") that are completely specific to each person. The estimated frequency of unrelated persons showing the same DNA fingerprint is extremely low—5×10^{-19}—and for siblings to share the same pattern is only 1×10^{-6}.

The possible applications of this test to various fields are immense. It opens up a novel approach in forensic science: bits of tissue, stains of blood, or other body fluids left at the scene of a crime may be used to identify their human source. The test has already been done by the Leicestershire police to identify a murderer in a group of several thousand suspects. A miniscule specimen is sufficient to yield a few micrograms of DNA, material whose stability confers an additional advantage for its use as a marker for identification. The state of California is planning a computerized data bank of DNA fingerprinting information on convicted criminals to facilitate the rapid identification of repeat offenders. The test is also conclusive for all practical purposes in resolving cases of controversial parenthood or other familial relationships. Recently, an immigration case concerning questionable maternity was settled by DNA fingerprinting, permitting the son's emigration to England. Routine DNA fingerprinting by immigration authorities should accelerate the application process and avoid cases of arbitrary judgment by immigration officials.

The test provides an unequivocal criterion for discriminating between monozygotic and dizygotic twins of the same sex at birth, until now a problematic area in genetic studies involving human twins. Other potential medical applications of the test include monitoring engraftment of donor marrow;